D. Laurence #26 9/16/02

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
YOSHIHIKO HIGUCHI ET AL.	Art Unit: 1743
Serial No. **09/473,165**	Examiner: **CROSS, LATOYA I.**
Filed: **DECEMBER 28, 1999**	
For: **DRY MEASURING TEST DEVICE**	

APPEAL BRIEF

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This Appeal Brief is filed in triplicate pursuant to 37 C.F.R. § 1.192 along with the fee set forth in 37 C.F.R. § 1.17(c) and appeals the respective decisions of the Examiner mailed September 11, 2001 and March 6, 2002. Although Applicants do not believe any extension fees are due, the Commissioner is hereby authorized to charge any additional fees required to Account No. 11-0855.

1. REAL PARTY IN INTEREST

The real party in interest is Kyoto Daiichi Kagaku Co., Ltd.

2. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

3. STATUS OF CLAIMS

Claims 6–8 and 10–13 are pending in the above-identified application. Claims 6–

I hereby certify that this correspondence is being deposited with the United States Postal Service, Express Mail Label No. EV041931068US addressed to: Assistant Commissioner for Patents, Washington, DC 20231, on September 11, 2001.

Jeffery B. Arnold - Reg. No. 39,540

8 and 10–13 are the subject of this appeal. A copy of Claims 6–8 and 10–13 is attached as Appendix A.

4. STATUS OF AMENDMENTS

During prosecution of the above-identified application, Claims 6 and 10-13 were amended. All amendments were entered, and there are no outstanding amendments.

5. SUMMARY OF THE INVENTION

The claimed invention is directed to a dry measuring test device comprising a reagent layer. The reagent layer comprises a reagent containing a chromogen and a matrix which retains the reagent in the form of a layer. A light blocking layer containing light blocking particles in the form of polymer beads embedding the light blocking particles is laminated on the reagent layer. A substance to be measured in a liquid sample is determined by the dry measuring test device by measuring the degree of coloring of the chromogen generated through the reaction between the substance to be measured and the reagent in terms of reflectance of light entered into the reagent layer.

6. ISSUES PRESENTED ON APPEAL

Are Claims 6–8 and 10–13 unpatentable under 35 U.S.C. § 103(a) over U.S. Patent No. 5,589,347 to Arai et al.?

7. GROUPING OF CLAIMS

The claims stand or fall together.

8. ARGUMENT

I. General Background.

Among the methods for determining a specific component in a liquid sample, in particular, the methods using a dry measuring test device such as test paper have been widely used at present for various purposes because the handling thereof is comparatively

easy. Such dry measuring test devices basically have a structure that comprises a reagent layer having a matrix, in which a liquid sample can penetrate and be developed, containing a reagent which is soluble in the above liquid sample and reactive with the substance to be measured to generate a signal such as color, light, or the like, which can be detected and determined by analytical machines.

Among the above-described dry measuring test devices, the dry measuring test devices that determine a substance to be measured in a liquid sample through the coloring reaction between the substance to be measured and the reagent usually quantifies the substance to be measured in the liquid sample by measuring an amount of a coloring matter which is colored through the reaction of the substance to be measured with the reagent in terms of reflectance of incident light in the reagent layer. In order to conduct accurate measurement, such dry measuring test devices based on the coloring reaction contain a reflector in the reagent layer as well as the reagent for coloring. For example, the dry measuring test device described in Japanese Examined Patent Publication No. 7-21455 has a reagent layer containing not only the reagent to be used for the measurement but also, as a reflector, light-reflective water-insoluble particles, for example, white pigments such as titanium oxide, zinc oxide, barium sulfate, magnesium oxide, or the like. When the sample is whole blood, these pigments sometimes play a role of preventing red blood cells which cause measurement errors from invading the reagent layer.

However, even if the reflector is contained in the reagent layer as described above, since the reagent layer must essentially have such a structure that a sample can easily penetrate and diffuse in the layer, more specifically, it must be as thin as possible and porous, if a large amount of white pigments are contained to improve the measurement accuracy, the reagent layer becomes so dense that a liquid sample hardly penetrates it because the particle

diameter of the white pigments is extremely small (0.1 to 0.3 μm). Accordingly, at the time of the measurement, penetration and development of the liquid sample proceeds slowly and it takes long time until the amount of the coloring matter generated by the reaction with the reagent becomes sufficient so as to be measurable. Thus, there is a problem in working performance.

Also, in the dry measuring test devices, a concentration condition of the reaction is considerably high, as the reagent is dissolved in the liquid sample to commence the reaction. Further, the matrix which constitutes the reagent layer usually has a structure that it easily becomes dry since its surface area contacting air is large. Although it does not matter very much when measurement is carried out by immersing the dry measuring test device in a comparatively large amount of the liquid sample, in the case of carrying out the measurement by spotting a small amount of the liquid sample, it is disadvantageous in that the measurement accuracy may possibly reduced by being affected by dryness if the measuring time is prolonged as described above.

Furthermore, if the amount of the white pigment is reduced to the extent that the reagent layer can have a porous structure, the liquid sample penetrates and is developed easily and the time required for the measurement can be shortened. However, such a reagent layer can keep high whiteness when it is dried, but light reflective efficiency becomes insufficient when the liquid sample penetrates the reagent layer, that is, at the time of measurement. As a result, the measurement is easily affected by transmission, absorption, and scattering of measuring light, or incidence of light from the outside of the test device, and the like. This is because, in the case where the reagent layer has a porous structure, an air layer incorporated therein raises light refractive index and diffused reflection light is increased, which makes apparent whiteness high when the layer is dried, while, when the

layer is moisturized, moisture incorporated in the layer lowers light refractive index and transmitted light increases more than diffused reflection light, thereby reducing apparent whiteness.

Particularly, when the liquid sample contains a coloring component, for example, blood cells or the like, the component causes absorption, scattering, and the like of measuring light even if it has penetrated into the reagent layer. Alternatively, the coloring component causes absorption, scattering, and the like of measuring light which has transmitted the reagent layer even if the component is present in the outside of the reagent layer. When the sample is whole blood, each sample gives a different amount of measuring light which enters and is reflected by the reagent layer because of difference in the hematocrit value or the like. When the dry measuring test device has a support or the like, measuring light which transmits the reagent layer is reflected by the support and the reflective light is entered into the reagent layer. This reflective light is unfavorably detected together with the light reflected from the reagent layer. Accordingly, the amount of the reflective light from the support or the like varies depending on, for example, the hematocrit value in the case of whole blood samples, which affects the measured values.

Under these circumstances, in order to realize speedy and highly accurate measurement using a dry measuring test device that determines a substance to be measured in a liquid sample by subjecting the substance to the coloring reaction and determining the degree of coloring in terms of reflectance, it has been desired to develop a method for obtaining sufficiently measurable reflectance corresponding to a low degree of coloring upon measurement of reflectance or a method for reducing influence of absorption and scattering of measuring light attributed to solid matter contained in the liquid sample and of external stray light entered from the opposite side of the surface of the light-measuring site, with

maintaining such a structure that the liquid sample penetrates the reagent layer of the dry measuring test device to generate a measurable amount of coloring matter within a short period of time, namely, that the measuring time is so short that influence of dryness can be reduced.

II. Claims 6–8 and 10–13 are patentable under 35 U.S.C. § 103(a) over U.S. Patent No. 5,589,347 to Arai et al.

The Examiner has rejected Claims 6–8 and 10–13 under 35 U.S.C. § 103(a) over U.S. Patent No. 5,589,347 to Arai et al. ("*Arai*"). Applicants respectfully assert that *Arai* does not suggest each and every element of Applicants' claimed invention, Applicants' claimed invention is not obvious, and Applicants' claimed invention is patentable. Reversal of the Examiner's rejection is therefore requested.

In general, the invention as claimed in Claims 6–8 and 10–13 is directed to a dry measuring test device comprising a reagent layer. The reagent layer comprises a reagent containing a chromogen and a matrix which retains the reagent in the form of a layer. A light blocking layer containing light blocking particles in the form of polymer beads embedding the light blocking particles is laminated on the reagent layer. A substance to be measured in a liquid sample is determined by the dry measuring test device by measuring the degree of coloring of the chromogen generated through the reaction between the substance to be measured and the reagent in terms of reflectance of light entered into the reagent layer.

According to the Examiner, *Arai* teaches a multi-layer analysis element containing at least one hydrophilic polymer layer and a spreading layer on the hydrophilic layer. It is asserted that the hydrophilic layer contains colorimetric reagents and serves as the reagent layer. Further, it is asserted that the multi-layer system also provides for a light-shielding layer, which contains light shielding particles such as carbon black, on the reagent layer.

The Examiner states that *Arai* discloses that the light shielding layer is formed of fine particles, *e.g.*, carbon black, dispersed in hydrophilic polymer binders such as polyvinyl

alcohol (col. 3, lines 1-5 and col. 4, lines 39-47). At col. 5, lines 4-15, *Arai* teaches that the ratio of hydrophilic polymer binder to light-shielding particles is about 2.5-7.5 to 10. *Arai* also discloses that single layers may be made to serve two or more functions (col. 3, lines 44-50).

However, the Examiner admits that *Arai* differs from the instantly claimed invention in that **the limitation of embedding the light-shielding particles in polymeric beads is not disclosed**. Yet, the Examiner states that *Arai* discloses that light shielding particles are dispersed into a polymer and coated onto the reagent layer to dry. From this and without support, the Examiner concludes that the dispersion of light shielding particles into the polymer is equivalent to embedding the particles in polymers, absent evidence to the contrary. The Examiner observed that Applicants state at page 34, lines 20-25, of the instant specification that light blocking particles embedded into polymeric beads are commercially available. Thus, the Examiner concluded that one of ordinary skill in the art would consider "embedded" particles and "dispersed" particles to be equivalent absent evidence of unexpected results, and it would be obvious to choose either in preparing the multi-layer test system.

In response, Applicants filed the Declaration Under 37 C.F.R. § 1.132 ("the Higuchi Declaration") by Yoshihiko Higuchi, one of the inventors of the above-identified application, which is attached hereto and made a part hereof as Appendix "B". As explained to the Examiner, the Higuchi Declaration demonstrates that by using polymer beads embedding carbon black, measurement can be shortened and the influence of hematocrit values on reflectance becomes small. As a result, measurements can be made more rapidly and accurately, compared with the direct use of carbon black. The advantages of embedding the light-blocking particles in polymer beads, over dispersing those particles into the polymer, are not suggested by Arai and one of ordinary skill could not find from that reference any teaching of those results or of the structural arrangement producing those results.

According to the Examiner, the Higuchi Declaration fails to show any substantial unexpected results. The Examiner asserts that Applicants' evidence shows a difference of only a

few seconds in using light blocking particles dispersed in a polymer, as described by *Arai*, as opposed to light blocking particles embedded in a polymer in accordance with the claimed invention. Further, the Examiner states that the influence in the difference of hematocrit values on reflectance when embedded particles are used, as shown by the third graph in the Higuchi Declaration, is quite small. The Examiner then concluded that the data presented in the Higuchi Declaration fails to show a substantial difference in using dispersed particles rather than embedded particles. Despite Applicants' explanation that the differences between the embedded light shielding particles and the dispersed light shielding particles are significant, the Examiner stated that the Higuchi Declaration seeks to explain the time difference in using embedded particles over dispersed polymers. The Examiner further stated that the time differences demonstrated in the Higuchi Declaration is an unclaimed limitation and is more involved with how the device is used. Respectfully, the Examiner has failed to appreciate that these time differences clearly demonstrate unexpected results.

The determination of obviousness under 35 U.S.C. § 103 is a legal conclusion based on factual evidence. *Burlington Indus., Inc. v. Quigg*, 822 F.2d 1581, 1584, 3 U.S.P.Q.2d 1436, 1439 (Fed. Cir. 1987). The prior art relied upon, coupled with the knowledge generally available in the art at the time of the invention, must contain some suggestion or incentive that would have motivated one of ordinary skill in the art to modify a reference or to combine references. *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). Further, the prior art reference or combination of references must teach or suggest all the limitations of the claims. See *In re Wilson*, 424 F.2d 1382, 1385, 165 U.S.P.Q. 494, 496 (C.C.P.A. 1970). To support a conclusion of obviousness, "either the references must expressly or impliedly suggest the claimed combination or the Examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *Ex parte Clapp*, 227 U.S.P.Q. 972, 973 (Bd. Pat. App. & Int. 1985). In evaluating obviousness, the Federal Circuit made it very clear that one must look to see if "the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have had a reasonable likelihood of success viewed in light of the prior

art." *In re Dow Chemical Co. v. American Cyanamid Co.*, 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). Further, "[i]t is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art." *In re Wesslau*, 353 F.2d 238, 241, 147 U.S.P.Q. 391, 393 (C.C.P.A. 1965). Applicants respectfully assert that the Examiner's line of reasoning is not convincing, because there is no suggestion to modify the light shielding layer of *Arai* formed of dispersed particles with light blocking particles embedded into polymeric beads. Further, the Higuchi Declaration clearly demonstrates that there is a difference between dispersed particles and light blocking particles embedded into polymeric beads in the claimed invention and such difference is unexpected.

As discussed above, the reagent layer of the claimed invention comprises a reagent containing a chromogen and a matrix which retains the reagent in the form of a layer. A light blocking layer containing light blocking particles in the form of polymer beads embedding the light blocking particles is laminated on the reagent layer. The Examiner admits that *Arai* differs from the instantly claimed invention in that **the limitation of embedding the light-shielding particles in polymeric beads is not disclosed**. According to the Examiner, *Arai* only discloses a dispersion of light shielding particles into a polymer film. There is no suggestion by *Arai* to form a light blocking layer containing light blocking particles in the form of polymer beads embedding the light blocking particles. Further, there is no significance that light blocking particles embedded into polymeric beads are commercially available. Commercial availability of a product does not in and of itself render its use obvious in an invention when considering the combination of all claim elements. Accordingly, *Arai* does not suggest each and every limitation of the claimed invention. For this reason alone, Applicants respectfully submit that Claims 6–8 and 10–13 are patentable under 35 U.S.C. § 103(a) over *Arai*.

Further, the Higuchi Declaration demonstrates that light blocking particles embedded in polymer beads is **not** the equivalent of dispersed light blocking particles. However,

the Examiner states that the declaration fails to show any substantial unexpected results. The Examiner stated as follows:

> "Applicant's evidence shows a difference of only a few seconds in using light blocking particles dispersed in a polymer (Arai et al '347) as opposed to light blocking particles embedded in a polymer. With respect to the hematocrit values, Applicants have argued that a lower influence of hematocrit values on reflectance results where embedded particles are used. Again, the difference in influences, as shown by the third graph in Applicant's declaration, is quite small."

The Examiner underestimates the significance of the difference shown in the Higuchi Declaration, as discussed below. The Higuchi Declaration shows that the measurement can be shortened. The difference is not a few seconds.

Appendix "C", which is attached hereto and made a part hereof, is a graph which is reproduced from the data shown in the Higuchi Declaration. In Appendix "C", data at Ht (hematocrit value) of 45% which is considered as a normal value are overlaid for facilitating the comparison. "CB" is a prior art device as disclosed by *Arai*. "MBX-5/Black" is a device according to the present invention. The reflectance at 20 seconds when the device according to the present invention is used is the same as that at 40 seconds when the prior-art device is used. In other words, 20 to 30 seconds after the detection is started, the measurement can be completed in the case of the device according to the present invention, while reaction still progresses in the case of the prior-art device.

The dry measuring test device is generally used for screening because of its simplicity. A large number of specimens are usually tested by the dry measuring test device. In this case, it is clear how halving the required measurement time contributes to improvement of

working performance. The Examiner fails to sufficiently consider what is desired by experts in this art. Reducing the 40-sec period to the 20-sec period (a 50% reduction) is remarkably significant in this art.

In the art of dry measuring test devices, there were many technological innovations to shorten the required measurement time. The shortening of the measurement time is the major problem in the development of dry measuring test devices. In fact, every time a manufacturer develops a new product, the required measurement time is shortened. Under these circumstances, halving of the measurement time achieved by the present invention is a remarkable and unexpected advantage. The Examiner's statement that the difference is only a few seconds is unreasonable in view of the nature of the art.

Also, tests using the dry measuring test device are used as "Point of Care" tests which are conducted at the patient's bedside. In this case, it is quite sure that the fact that the measurement is completed in half the usual time (that is, results are obtained more rapidly) compared with the prior art, remarkably relieves an inpatient from anxiety and stress. The shortening of the measurement time will result in shortening of the hospitalization period.

In addition, the shortening the measurement time contributes to improvement of accuracy and sensitivity of the measurement as discussed below.

As seen from the graph of the dry measuring test device shown in Appendix "C", in the time course of the prior-art dry measuring test device (CB), the point at which the curve begins to level is 60 seconds after the start of detection. This means that reaction between a reagent and a sample is substantially complemented after 60 seconds.

On the other hand, in the time course of the dry measuring test device of the present invention (MBX-5/Black), the point at which the curve begins to level is 30 seconds after

the start of detection. This mean that reaction between a reagent and a sample is substantially completed after 30 seconds.

If the measurement is based on the end-point method, no result can be obtained before the completion of the reaction. That is, measurement can not be made before the completion of the reaction. In this connection, the shorter is the measurement time, the lesser is dryness of a sample. This is very advantageous feature in the dry measuring test device. In the case of the dry measuring tests device on which a small amount (e.g., one drop) of a sample is often provided, the measurement accuracy is largely affected by dryness caused by the difference of tens of seconds. See page 3, line 14 to page 4, line 1 of the specification of the above-identified application. The difference in tens of seconds is **not** negligible.

By using polymer beads embedding carbon black, the penetration rate of a sample is improved. That is, the development of the sample rapidly occurs in both the vertical direction and the horizontal direction. Thus, the development of the sample is efficiently conducted. Because reaction between a reagent and a sample uniformly occurs by this feature, it becomes free from unevenness, which results in good accuracy. Also, the improvement of the penetration rate results in high sensitivity. If penetration of the sample is slow, the reaction between the reagent and the sample tends to be retarded. To the contrary, if penetration of the sample is fast, the sample is smoothly supplied to the reagent, whereby the reaction occurs under good conditions. Thus, high sensitivity is obtained.

As discussed above, the difference of tens of seconds is important and unexpected in the art. Furthermore, as discussed in the previous response, the declaration shows that the influence of hematocrit values becomes small.

In Appendix "D", which is attached hereto and made a part hereof, the upper graph is the original graph in the Higuchi Declaration, and the lower graph is a graph reproduced by changing the Y axis from the reflectance to the glucose concentration. The concentration is

calculated from the reflectance when the reaction is completed. That is, for calculation of the concentration, the reflectance at 40 seconds is used in the case of the prior art dry measuring test device (CB) and the reflectance at 20 seconds is used in the case of the dry measuring test of the present invention (MBX-5/Black). The standard curves for the calculation are shown in Appendix "E", which is attached hereto and made a part hereof.

In the lower graph of Appendix "D", the Y axis is shown in the glucose concentration (mg/dl). As seen from this graph, the concentrations measured with the dry measuring test device of the present invention (MBX-5/Black) do not go out of the range of 150 to 200 mg/dl regardless of the hematocrit values. On the other hand, the concentrations measured with the prior-art dry measuring test device (CB) vary between 100 and 230 mg/dl because they are largely influenced by the hematocrit values. When the prior art dry measuring device is used, the measured blood sugar value may be different from the true valuably over 10 mg/dl. The measurement error is very significant in the clinical test for blood sugar.

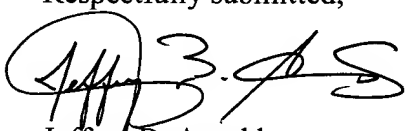
The Examiner underestimates the difference in the reflectance because how the clinical data is calculated from the reflectance is not considered. As discussed above, the difference in the reflectance shown in the declaration is significant in the art. The Examiner's statement that the difference is quite small is unreasonable in view of the nature of the art. Further, such differences clearly indicate that light blocking particles embedded in polymer beads is **not** the equivalent of dispersed light blocking particles and such differences are significant. Respectfully, Applicant's have demonstrated substantial unexpected results by the Higuchi Declaration. Accordingly, Applicants respectfully submit that Claims 6–8 and 10–13 are patentable under 35 U.S.C. § 103(a) over *Arai*.

In view of the above, the rejection of Claims 6–8 and 10–13 should be reversed.

CONCLUSION

It is respectfully submitted that Claims 6–8 and 10–13 are patentable under 35 U.S.C. § 103(a) over *Arai*. Further, it is respectfully submitted that *Arai* does not suggest each and every element of the invention as claimed in Claims 6–8 and 10–13. Therefore, it is requested that the rejections of Claims 6–8 and 10–13 under 35 U.S.C. § 103(a) over *Arai* by the Examiner be reversed.

Respectfully submitted,



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(KS#45455-239355)

APPENDIX A

Claims involved in the appeal:

6. A dry measuring test device, comprising a reagent layer comprising a reagent containing a chromogen and a matrix which retains said reagent in the form of a layer, for determining a substance to be measured in a liquid sample by measuring the degree of coloring of the chromogen generated through the reaction between the substance to be measured and the reagent in terms of reflectance of light entered into the reagent layer, wherein a light blocking layer containing light blocking particles in the form of polymer beads embedding the light blocking particles is laminated on the reagent layer.

7. The dry measuring test device as claimed in Claim 6, wherein the light blocking particles are selected from the group consisting of carbon black, iron (II) oxide, iron (III) oxide, phthalocyanine blue, and phthalocyanine green.

8. The dry measuring test device as claimed in Claim 6, wherein the light blocking particles are contained in an amount of 15 to 90 wt% based on the total weight of the light blocking layer.

10. The dry measuring text device as claimed in Claim 6, wherein said polymer beads contain as a main component a compound selected from the group consisting of: polymer or copolymer having as a main component monomers selected from the group consisting of acrylic acid, methacrylic acid, maleic acid, ester of these substances, styrene, and alkylstyrene; polyurethane; polyurea; polyethylene; polypropylene; and polyvinyl chloride.

11. The dry measuring test device as claimed in Claim 6, wherein the light blocking particles are contained in an amount of 10 to 70 w/v% based on the total content of the polymer beads, and the polymer beads are contained in the light blocking layer in an amount of 30 to 90wt% based on the total weight of the light blocking layer.

12. The dry measuring device as claimed in Claim 6, wherein an average particle diameter of the polymer beads ranges from 1 to 40 μm.

13. The dry measuring test device as claimed in Claim 6, wherein the reagent layer further contains polymer beads embedding the light reflective particles.

APPENDIX B

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

Y. HIGUCHI ET AL. : GROUP ART UNIT: 1743

SERIAL NO.: 09/473,165 :

FILED: DECEMBER 28, 1999 : EXAMINER: CROSS, L.

FOR: DRY MEASURING TEST DEVICE

DECLARATION UNDER 37 C.F.R. §1.132

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231
SIR;

I, Yoshihiko Higuchi, a citizen of Japan, one of the inventors of the above-identified application, of 4-18-15 Seikadai, Seika-machi, Soraku-gun, Kyoto-fu, Japan, hereby declare and state that:

1. I received a Bachelor of Engineering degree in 1989 from the Department of Applied Chemistry, Faculty of Engineering, Osaka Institute of Technology.

2. I have been employed by Arkray, Inc. (former Kyoto Daiichi Kagaku Co., Ltd.) since March, 1989.

3. I am engaged in research on development of test strips for clinical chemistry.

4. The following experiments were conducted by me or under my direct supervision.

Methods and results:

[Preparation of dry measuring test device]

I. Dry measuring test device 1 (Present invention)

Components for a reagent layer were mixed according to the composition of the dry measuring test device 1 shown in the following table to prepare a coating liquid for the reagent layer. Cell Guard (Hoechst Cellanese) was attached on a glass palte so

as to form no wrinkle. The coating liquid for the reagent layer was applied to the resulting Cell Guard by using a knife coater at a thickness of 100 μm and dried at 25°C and at a humidity of 15% for 30 minutes to form a reagent layer. Further, components for a light blocking layer were mixed according to the composition of the dry measuring test device 1 shown in the following table were mixed to prepare a coating liquid for the light blocking layer. The coating liquid for the light blocking layer was applied to the reagent layer by using a knife coater at a thickness of 40 μm and dried at 25°C and at a humidity of 15% for 30 minutes to form a light blocking layer. Then the Cell Guard, on which the reagent layer on which the light blocking layer was laminated ("light blocking layer/reagent layer) was provided, was peeled from the glass plate and cut into the size of 7 mm x 7 mm. The resulting light blocking layer/reagent layer on Cell Guard having a size of 7 mm x 7 mm square was attached by heat press on a PET film having a size of 30 mm x 7 mm with a hole of a diameter of 4 mm so that the side of Cell Guard could face the PET film to cover the hole. A cover of a thermoplastic resin was attached on the light blocking layer/reagent layer side of the PET film so as to form the capillary compartment between the cover and the PET film. The cover had a liquid sample-supplying hole and an air hole. Thus, a dry measuring test device of which light blocking layer contains polymer beads embedding carbon black (dry measuring test device 1) was prepared.

II. Dry measuring test device 2 (Comparative Experiment)

A dry measuring test device was prepared in the same manner as the dry measuring test device 1 except that components for a reagent layer and a light blocking layer were mixed according to the compositions of the dry measuring test device 2 shown in the following table. Thus, a dry measuring test device in which carbon black were directly distributed in the layer (dry measuring test device 2) was prepared.

	Dry measuring test device 1 (Present invention)		Dry measuring test device 2 (Comparative experiment)	
	Reagent layer	Light blocking layer	Reagent layer	Light blocking layer
Borate buffer (150 mM, pH 7.0)	29.0 g	-	29.0 g	-
Hydroxypropyl cellulose	1.3 g	1.3 g	1.3 g	1.3 g
Techpolymer MBX-5 (White)*1	5.0 g	-	-	-
Techpolymer MBX-5 (Black)*2	-	5.0 g	-	-
Titanium dioxide particles	-	-	2.5 g	-
Carbon black	-	-	-	2.5 g
Propiofan (BASF)	1.3 g	1.3 g	1.3 g	1.3 g
TES buffer (300 mM, pH 7.0)	5.0 g	5.0 g	5.0 g	5.0 g
Tween-20 (50 wt%)	3.2 g	3.2 g	3.2 g	3.2 g
Glucose oxidase	138 kU	-	138 kU	-
Peroxidase	103 kU	-	103 kU	-
4-Aminoantipyrine	0.2 g	-	0.2 g	-
MAOS (Dojin)	0.5 g	-	0.5 g	-
Distilled water	2.4 g	38.6 g	2.4 g	38.6 g

*1 manufactured by Sekisui Kaseihin Kogyo; content of titanium dioxide: 50 wt%

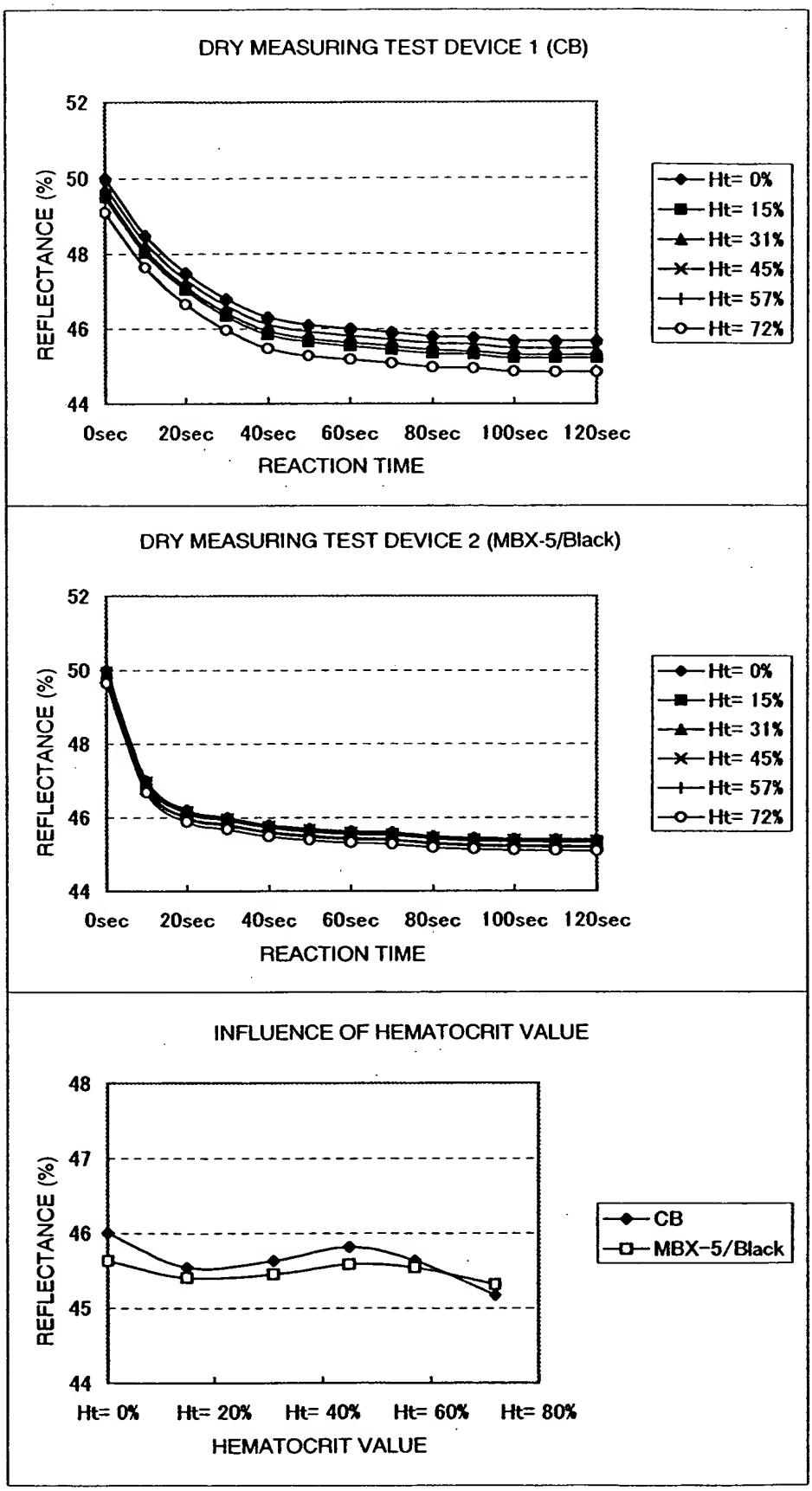
*2 manufactured by Sekisui Kaseihin Kogyo; content of carbon black: 50 wt%

The compositions were adjusted so that content of carbon black in the light blocking layer became the same between the dry measuring test devices 1 and 2.

[Evaluation of dry measuring test device]

The dry measuring test devices prepared in the above were tested for influence of the difference of the hematocrit value of the whole blood sample on the measured value.

Whole blood (glucose concentration: 99 mg/dl) supplemented with a glycolysis inhibitor (NaF) was adjusted to have different hematocrit values (0%, 15%, 31%, 46%, 57% and 71%) to prepare six samples. 10 μl of each sample was spotted on the reagent layer of the dry measuring test device of the present invention. From 5 seconds later, light of 640 nm was irradiated from the side of Cell Guard through the measuring light-irradiation hole and obtained reflectance was measured using a reflectiometer (color-difference meter). The same test was carried out for the comparative dry measuring test device.
The results are shown below.


DRY MEASURING TEST DEVICE 1 (CB)
52
50
48
46
44
REFLECTANCE (%)
0sec
20sec
40sec
60sec
80sec
100sec
120sec
REACTION TIME
Ht= 0%
Ht= 15%
Ht= 31%
Ht= 45%
Ht= 57%
Ht= 72%
DRY MEASURING TEST DEVICE 2 (MBX-5/Black)
52
50
48
46
44
REFLECTANCE (%)
0sec
20sec
40sec
60sec
80sec
100sec
120sec
REACTION TIME
Ht= 0%
Ht= 15%
Ht= 31%
Ht= 45%
Ht= 57%
Ht= 72%
INFLUENCE OF HEMATOCRIT VALUE
48
47
46
45
44
REFLECTANCE (%)
Ht= 0%
Ht= 20%
Ht= 40%
Ht= 60%
Ht= 80%
HEMATOCRIT VALUE
CB
MBX-5/Black

The upper graph shows the time-cource of reflectance in the measurement using the dry measuring test device 2 in which carbon black were directly distributed in the light blocking layer. At any of hematocrit values, the refelectance gradually decreased. This indicates that sinking of the sample into the reagent layer is slow.

The middle graph shows the time-cource of reflectance in the measurement using the dry measuring test device 1 in which polymer beads embedding carbon black were contained in the light blocking layer. At any of hematocrit values, the reflectance sharply decreased at the beginning. This indicates that sinking of the sample into the reagent layer is fast, in other words, the reaction proceeds fast. Because the rection proceeds fast, it is possible to shorten a measurment time.

The lower graph shows comparison of reflectance at 60 seconds between the dry measuring test devices 1 and 2. When polymer beads embedding carbon black was used, influence of hematocrit values on reflectance became small, compared with when carbon black was directly used.

Conclusion

By using polymer beads embedding carbon black, a measurement can be shortened and influence of hematocrit values on reflectance becomes small, whereby measurement can be made rapidly and accurately, compared with the direct use of carbon black.

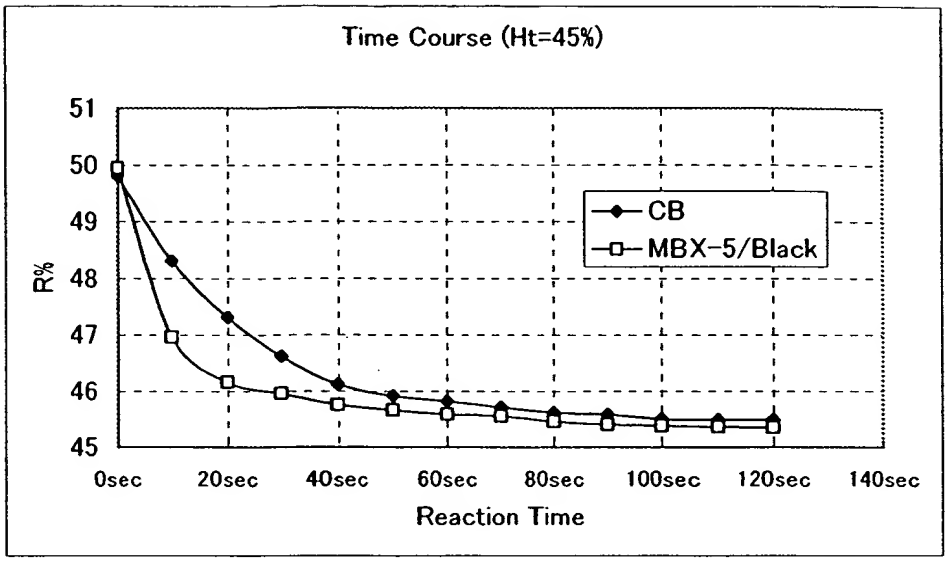
I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: June 15, 2001

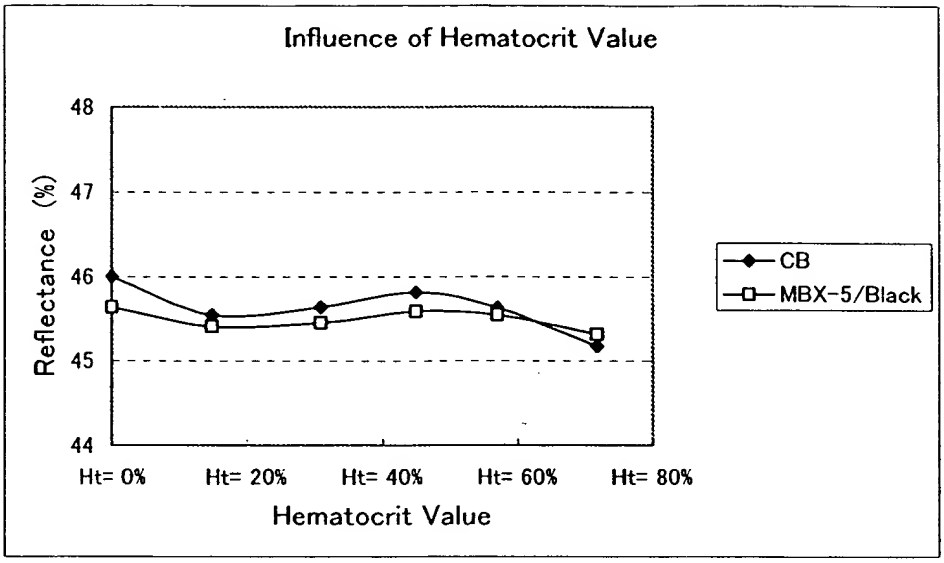
Yoshihiko Higuchi

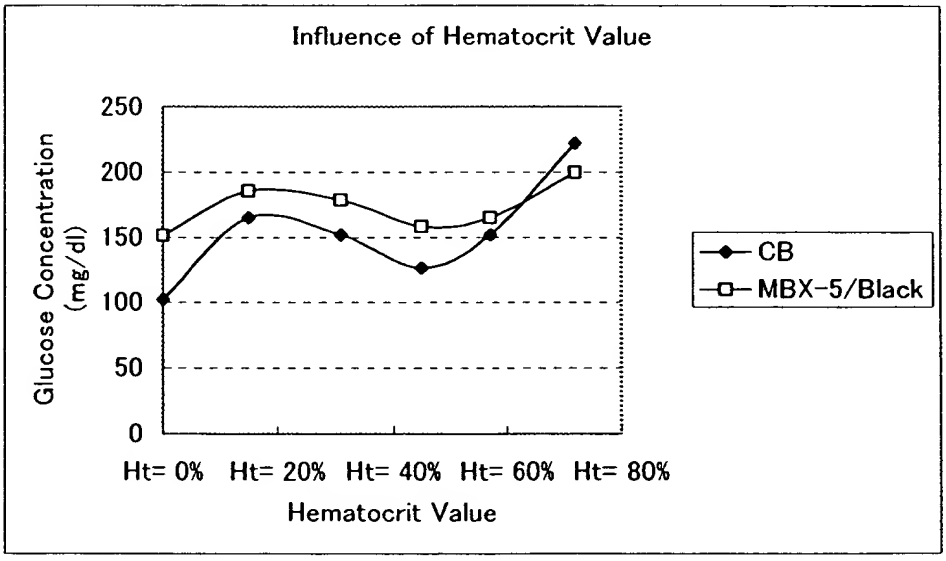
Yoshihiko Higuchi

APPENDIX C

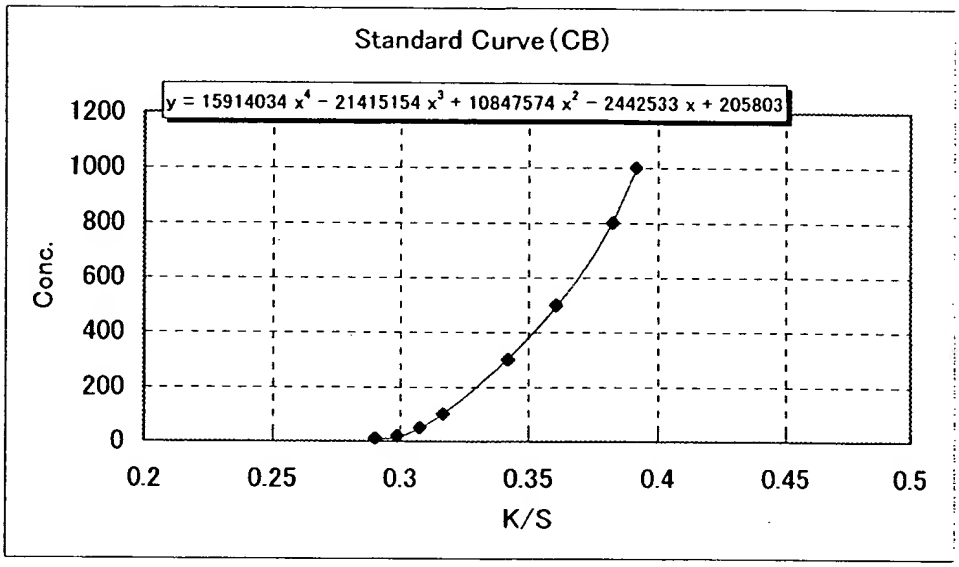

Time Course (Ht=45%)
51
50
49
48
47
46
45
R%
CB
MBX-5/Black
0sec
20sec
40sec
60sec
80sec
100sec
120sec
140sec
Reaction Time

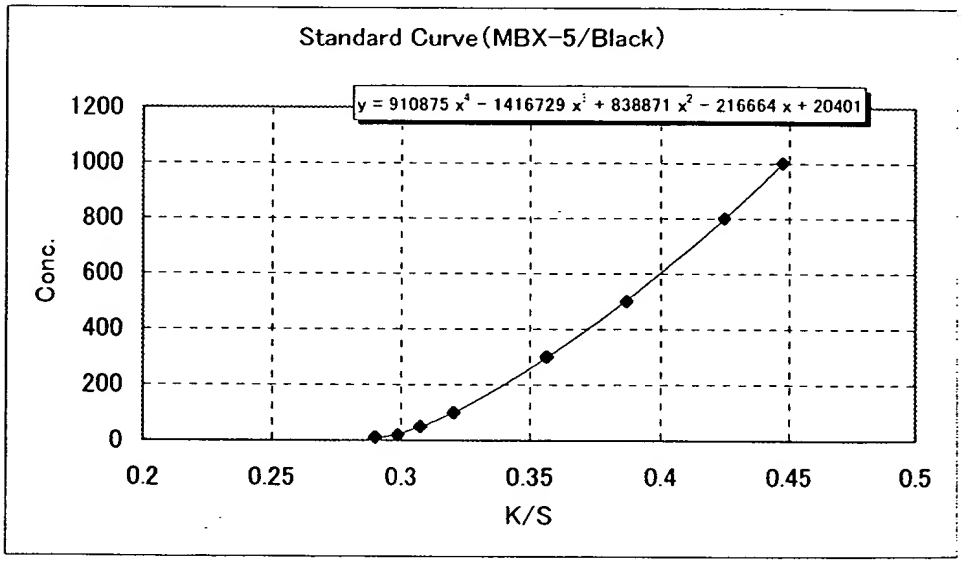
APPENDIX D


Influence of Hematocrit Value
48
47
46
45
44
Reflectance (%)
Ht= 0%
Ht= 20%
Ht= 40%
Ht= 60%
Ht= 80%
Hematocrit Value
CB
MBX-5/Black


Influence of Hematocrit Value
250
200
150
100
50
0
Glucose Concentration (mg/dl)
Ht= 0%
Ht= 20%
Ht= 40%
Ht= 60%
Ht= 80%
Hematocrit Value
CB
MBX-5/Black

APPENDIX E


Standard Curve (CB)
y = 15914034 x⁴ - 21415154 x³ + 10847574 x² - 2442533 x + 205803
1200
1000
800
600
400
200
0
Conc.
0.2
0.25
0.3
0.35
0.4
0.45
0.5
K/S


Standard Curve (MBX-5/Black)
y = 910875 x⁴ - 1416729 x³ + 838871 x² - 216664 x + 20401
1200
1000
800
600
400
200
0
Conc.
0.2
0.25
0.3
0.35
0.4
0.45
0.5
K/S